FORM-PTO-1390 (Rev. 9-2001) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER 022701-966
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U S APPLICATION NO (If known, see 37 C F R 1 5) UNASSIGNED 10/018786
INTERNATIONAL APPLICATION NO. PCT/FR00/01725	INTERNATIONAL FILING DATE 21 JUNE 2000	PRIORITY DATE CLAIMED 22 JUNE 1999
TITLE OF INVENTION AVIRULENT STRAINS OF XANTHOMONAS CAMPESTRIS WHICH PRODUCE XANTHAN		
APPLICANT(S) FOR DO/EO/US Jérôme PIERRARD et al.		

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:

Form PCT/IB/306; Form PCT/IB/308; Form PCT/IB/332; Form PCT/IPEA/416; Form PCT/IPEA/409; (2) Pages of Amended Sheets; (2) Sheets of Drawings (Figs. 1-3); (3) Pages of Sequence Listing and International Search Report.

21839

JC03 Rec'd PCT/PTO 21 DEC 2001

U.S. APPLICATION NO (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
UNASSIGNED 10/018786	PCT/FR00/01725	022701-966

		CALCULATIONS	PTO USE ONLY
21. ☒ The following fees are submitted:			
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .... $1,040.00 (960) International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .... $890.00 (970) International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .... $740.00 (958) International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) .... $710.00 (956) International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00 (962)			
ENTER APPROPRIATE BASIC FEE AMOUNT =		$ 890.00	
Surcharge of **$130.00 (154)** for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)).	20 ☐ 30 ☐	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	19 -20 =	0	X$18.00 (966)	$ 0.00	
Independent Claims	7 -3 =	4	X$84.00 (964)	$ 336.00	
Multiple dependent claim(s) (if applicable)			+ $280.00 (968)	$ 0.00	

TOTAL OF ABOVE CALCULATIONS =	$ 336.00	
Reduction for 1/2 for filing by small entity, if applicable (see below). +	$	-
SUBTOTAL =	$ 1,226.00	
Processing fee of **$130.00 (156)** for furnishing the English translation later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(f)). +	$	
TOTAL NATIONAL FEE =	$ 1,226.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 (581)** per property +	$	
TOTAL FEES ENCLOSED =	$ 1,226.00	
	Amount to be refunded:	$
	charged:	$

a. ☐ Small entity status is hereby claimed.

b. ☒ A check in the amount of $ 1,226.00 to cover the above fees is enclosed.

c. ☐ Please charge my Deposit Account No. 02-4800 in the amount of $______ to cover the above fees. A duplicate copy of this sheet is enclosed.

d. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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TERESA STANEK REA
NAME

30,427
REGISTRATION NUMBER

DECEMBER 21, 2001
DATE

Patent
Attorney's Docket No. 022701-966

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
Jérôme PIERRARD *et al.*	)	Group Art Unit: Unassigned
Application No.: Unassigned	)	Examiner: Unassigned
International Filing Date: 21 JUNE 2000 (Corresponds to PCT/FR00/01725)	)	
For: AVIRULENT STRAINS OF XANTHOMONAS CAMPESTRIS WHICH PRODUCE XANTHAN	)	

PRELIMINARY AMENDMENT

BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the above-captioned application as follows:

IN THE CLAIMS:

Kindly amend claims 2-19 as follows:

2. (Amended) The bacterial strain as claimed in claim 1, which has been made stably nonphytopathogenic by inactivation of at least one gene, of the *hrp* or *hrc* gene group.

3. (Amended) The bacterial strain as claimed in claim 1, which has been made stably nonphytopathogenic by inactivation of 5 to 9 genes of the *hrp* or *hrc* gene group.

4. (Amended) The bacterial strain as claimed in claim 1, which is a *Xanthomonas* strain which has lost the phytopathogenic nature by inactivation of at least one virulence gene, and which has conserved the ability to produce exopolysaccharide.

5. (Amended) The *Xanthomonas* strain as claimed in claim 4, which is of the species *Xanthomonas campestris*.

6. (Amended) The *Xanthomonas* strain as claimed in claim 5, which is *Xanthomonas campestris pv campestris*.

7. (Amended) The *Xanthomonas* strain as claimed in claim 1, wherein the inactivation of said gene(s) is obtained by deletion of a region of DNA of at least 1 kb, in the *hrp* or *hrc* gene group, and it conserves the ability to produce exopolysaccharide.

8. (Amended) The *Xanthomonas* strain as claimed in claim 1, which comprises a deletion of a region of DNA of at most 40 kb.

9. (Amended) The *Xanthomonas* strain as claimed in claim 8, which is obtained by deletion of all or part of the *hrp A1* to *hrpC2* genes.

10. (Amended) An essentially nonphytopathogenic *Xanthomonas* strain comprising a deletion of a region of DNA of at least 1 kb, in the *hrp* or *hrc* gene group, and it conserves the ability to produce exopolysaccharide.

11. (Amended) An essentially nonphytopathogenic *Xanthomonas* strain, obtained by deletion of all or part of the *hrp A1-C2* genes.

12. (Amended) An essentially nonphytopathogenic *Xanthomonas campestris* strain, selected from the group consisting of BIOCAT 1016, BIOCAT 1017, BIOCAT 1019, BIOCAT 1021 and BIOCAT 1022 strains, deposited at the CBS under the numbers CBS 101940, CBS 101941, CBS 101942, CBS 101943 and CBS 101944, respectively.

13. (Amended) The *Xanthomonas* strain as claimed in claim 4, wherein the exopolysaccharide is a xanthan gum.

14. (Amended) A method for preparing the strain as claimed in claim 9, comprising using a pRPA-BCAT 140 plasmid.

15. (Amended) A method for preparing a strain as claimed in claim 7, wherein the strain is obtained by homologous recombination with a plasmid comprising a deletion of all or part of the *hrp* or *hrc* genes.

16. (Amended) A method for preparing bacterial exopolysaccharide, comprising culturing a bacterial strain as claimed in claim 1 under conditions which allow the production of exopolysaccharide in the fermentation medium.

17. (Amended) A nucleic acid comprising the nucleotide sequence SEQ ID No. 3.

18. (Amended) A nucleic acid comprising the nucleotide sequence SEQ ID No. 6.

19. (Amended) A nucleic acid comprising the nucleotide sequence SEQ ID No. 7.

Application No. Unassigned
Attorney's Docket No. 022701-966

REMARKS

Entry of the foregoing amendments are respectfully requested.

Should the Examiner have any questions concerning the subject application, a telephone call to the undersigned would be appreciated.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: ______________________________
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Date: December 21, 2001

Attachment to Preliminary Amendment dated December 21, 2001
Mark-up of Claims 2 - 19

2. (Amended) The bacterial strain as claimed in claim 1, [characterized in that it] which has been made stably nonphytopathogenic by inactivation of at least one gene, [advantageously at least two genes, preferably at least three genes,] of the *hrp* or *hrc* gene group.

3. (Amended) The bacterial strain as claimed in claim 1 [or claim 2], [characterized in that it] which has been made stably nonphytopathogenic by inactivation of 5 to 9 genes of the *hrp* or *hrc* gene group.

4. (Amended) The bacterial strain as claimed in claim 1, [characterized in that it] which is a *Xanthomonas* strain which has lost the phytopathogenic nature by inactivation of at least one virulence gene, and which has conserved the ability to produce exopolysaccharide.

5. (Amended) The *Xanthomonas* strain as claimed in claim 4, [characterized in that it] which is of the species *Xanthomonas campestris*.

6. (Amended) The *Xanthomonas* strain as claimed in claim 5, [characterized in that it] which is *Xanthomonas campestris pv campestris*.

Attachment to Preliminary Amendment dated December 21, 2001
Mark-up of Claims 2 - 19

7. (Amended) The *Xanthomonas* strain as claimed in [any one of the preceding claims] claim 1, [characterized in that] wherein the inactivation of said gene(s) is obtained by deletion of a region of DNA of at least 1 kb, [preferably at least 3 kb, advantageously at least 5 kb,] in the *hrp* or *hrc* gene group, and [in that] it conserves the ability to produce exopolysaccharide.

8. (Amended) The *Xanthomonas* strain as claimed in [any one of the preceding claims] claim 1, [characterized in that] which comprises a deletion of a region of DNA of at most 40 kb.

9. (Amended) The *Xanthomonas* strain as claimed in claim 8, [characterized in that] which is obtained by deletion of all or part of the *hrp A1* to *hrpC2* genes.

10. (Amended) An essentially nonphytopathogenic *Xanthomonas* strain[, characterized in that it comprises] comprising a deletion of a region of DNA of at least 1 kb, [preferably at least 3 kb, advantageously at least 5 kb,] in the *hrp* or *hrc* gene group, and [in that] it conserves the ability to produce exopolysaccharide.

11. (Amended) An essentially nonphytopathogenic *Xanthomonas* strain, [characterized in that it is] obtained by deletion of all or part of the *hrp A1-C2* genes.

Attachment to Preliminary Amendment dated December 21, 2001
Mark-up of Claims 2 - 19

12. (Amended) An essentially nonphytopathogenic *Xanthomonas campestris* strain, [chosen] selected from the group consisting of BIOCAT 1016, BIOCAT 1017, BIOCAT 1019, BIOCAT 1021 and BIOCAT 1022 strains, deposited at the CBS under the numbers CBS 101940, CBS 101941, CBS 101942, CBS 101943 and CBS 101944, respectively.

13. (Amended) The *Xanthomonas* strain as claimed in [one of claims 4 to 13] claim 4, [characterized in that] wherein the exopolysaccharide is a xanthan gum.

14. (Amended) A method for preparing the strain as claimed in claim 9, comprising using a pRPA-BCAT 140 plasmid[, used for manufacturing the strain as claimed in claims 9 to 13].

15. (Amended) A method for preparing a strain as claimed in [any one of claims 7 to 13] claim 7, [characterized in that] wherein the strain is obtained by homologous recombination with a plasmid comprising a deletion of all or part of the *hrp* or *hrc* genes.

16. (Amended) A method for preparing bacterial exopolysaccharide, [in particular xanthan gum, characterized in that] comprising culturing a bacterial strain[, where appropriate of the *Xanthomonas* genus, preferably of the species *Xanthomonas*

Attachment to Preliminary Amendment dated December 21, 2001
Mark-up of Claims 2 - 19

campestris] as claimed in [any one of claims 1 to 13] claim 1 [is cultured] under conditions which allow the production of exopolysaccharide in the fermentation medium.

17. (Amended) A nucleic acid[, characterized in that it comprises] comprising the nucleotide sequence SEQ ID No. 3.

18. (Amended) A nucleic acid[, characterized in that it comprises] comprising the nucleotide sequence SEQ ID No. 6.

19. (Amended) A nucleic acid[, characterized in that it comprises] comprising the nucleotide sequence SEQ ID No. 7.

AVIRULENT STRAINS OF XANTHOMONAS CAMPESTRIS WHICH PRODUCE XANTHAN

The invention relates to novel bacterial strains, especially strains of *Xanthomonas*, in particular *Xanthomonas campestris*, which have lost the phytopathogenic nature but which have substantially conserved the ability to produce exopolysaccharide, in particular xanthan gum.

Xanthomonas campestris pv. *campestris* is a phytopathogenic Gram-negative bacterium of Crucifers which is used for the industrial production of xanthan gum (Martin, 1994, Res. Microbiol. 145:9 93-97).

The economic importance of this exopolysaccharide gives rise to many studies concerning the genes involved in this synthesis (Martin, 1994, mentioned above).

Many determinants of pathogenicity have been described (Dow and Daniels, 1994, In bacterial pathogenesis of plants and animals, JL Dangl, ed. Springer Verlag, Heidelberg). Among these, are extracellular enzymes with hydrolytic activity on plant tissues. When the secretion system responsible for exporting these enzymes is inactivated, strains of *X. campestris* have a nonphytopathogenic phenotype which is associated with with very reduced symptoms in the plants (Dow and Daniels, 1994, mentioned above). Among

the determinants of pathogenicity described is exopolysaccharide, which appears to have a role in the early phase of the disease (Dow and Daniels, 1994, mentioned above; Katzen *et al.*, 1998, J. Bacteriol. 180: 1607-1617). Similarly, an *hrpXc* gene, described in *X. campestris* pv. *campestris* (Kamoun *et al.*, 1992, Mol. Plant Microbe Interact. 5: 22-33), is involved in suppressing the defense responses of the compatible host plant, since the mutation of this gene leads to a characteristic necrotic reaction (hypersensitivity response, HR). The avirulence genes described in the various pathovars of *X. campestris* are also involved in the pathogenicity of the bacteria since they are recognized by plants which have the resistance gene corresponding and leading to an HR reaction (Dow and Daniels, 1994, mentioned above; Yang *et al.*, 1995, Mol. Plant Microbe Interact. 8: 627-631). Among the other genes involved in the pathogenicity of *Xanthomonas* (Dow and Daniels, 1994, mentioned above), two of the genes have been described in *X. campestris* pv *campestris*, mutations of which lead to reduced pathogenicity without the levels of accumulation of extracellular enzymes and of exopolysaccharides being modified (Osbourn *et al.*, 1990, Mol. Plant Microbe Interact. 3: 280-285). Other determinants of pathogenicity consist of various independent sets of genes which regulate the

synthesis of extracellular enzymes and of exopolysaccharide, among which are: the *rpfA* to *H* genes, mutations of which lead to a decrease in the production of exopolysaccharide; the *rpfN* gene, a repressor of the synthesis of these enzymes and of exopolysaccharide; the *clp* gene, mutations of which lead to reduced pathogenicity and to decreased production of exopolysaccharides (Dow and Daniels, 1994, mentioned above). Finally, other determinants of pathogenicity consist of the *hrp* genes.

The *hrp* (hypersensitivity reaction and pathogenicity) genes are essential for the pathogenicity concerning a compatible plant and for the hypersensitivity reaction concerning resistant hosts (Alfano and Collmer, 1997, J. Bacteriol. 179: 5655-5662). They have been cloned and characterized to diverse degrees in several phytopathogenic bacteria of the *Erwinia, Pseudomonas, Ralstonia* and *Xanthomonas* genera, in which they are relatively conserved (Zurek and Bukowski, 1998, Acta Microbiologica Polonica, 47: 227-241; Alfano and Collmer, mentioned above), in particular in *X. campestris* pv. *vesicatoria* (Huguet *et al.*, 1998, Molec. Microbiol 29: 1379-1390; Fenselau *et al.*, 1992, Molecular Plant-Microbe Interactions, 5: 390-396; Bonas, 1994, mentioned above). The most conserved among them have, moreover, been renamed *hrc*

genes (Bogdanove *et al.*, 1996, Mol. Microbiol., 20: 681-683). Among the functions of the *hrp* genes described to date are the regulation of their expression, the production of proteins which elicit the host's response, the constitution of a specific ("type III") secretion system and the synthesis of periplasmic glucans (Zurek et Bukowski, 1998, Acta Microbiologica Polonica, 47: 227-241; Mudgett et Staskawicz, 1998, Current Opinion in Microbiology 1: 109-114; Lindgren, 1997, Annu Rev. Phytopathol. 35: 129-152; Alfano and Collmer, 1997, mentioned above; Bonas, 1994, mentioned above). A set of *hrp* genes has been cloned in *X. campestris* pv. *campestris* (Arlat *et al.*, 1991, Mol. Plant Microbe Interact 4: 593-601) but not sequenced. It has also been reported that strains which carry mutations in these genes, produced by virtue of a transposon, are thought to have a normal production of exopolysaccharide, according to the appearance of the colonies on a dish. No more precise quantification of the xanthan productivity of these strains has, however, been published.

In addition, the mutations produced in these strains are not sufficiently stable in nature for industrial use for the production of xanthan gum. Specifically, the transposon used contains the gene encoding transposase (Simon *et al.*, 1989, Gene 80: 161-

169), which does not exclude an event of excision of the transposon at a frequency which may be estimated at between 10^{-6} and 10^{-3} per generation (Berg *et al.,* 1989, In Berg and Howe ed., Mobile DNA, American Society for Microbiology, Washington D.C. pp 879-926; Craig, In *Escherichia coli* and *Salmonella, Neidhardt ed.,* ASM Press, Washington, D.C. pp 2339-2362). In addition, the transposon used contains a gene for resistance to the antibiotics neomycin and kanamycin. Finally, the transposon inserted into the genome of these strains constitutes a DNA element which is nonhomologous since it is not a natural element of the genome of the strain used.

Although, at the current time in Europe, there is no specific regulation imposed by the phytopathogenic nature of *Xanthomonas campestris* pv. *campestris*, it is highly desirable, for reasons related to the environment, to use nonphytopathogenic strains of *Xanthomonas campestris*, in order to decrease the possible risk of contamination of cultures of agronomic interest close to the site. Selecting such a strain using conventional techniques of random mutagenesis for production is a long and tedious process since it must involve high throughput screening for isolating a strain which is nonphytopathogenic but which has conserved its productivity characteristics, i.e. with

no secondary mutations.

Moreover, the use of a genetically modified strain which produces a modified xanthan gum (as described in US 5,514,791) or which has improved productivity is subject to strict regulation (Theilleux 1998, Dictionnaire permanent Bioéthique et Biotechnologies [Permanent dictionary of bioethics and biotechnology], ed Législatives [legislative ed], pp 1595-1648). The latter imposes, in particular for a construct produced in a strain presenting a danger to plants, the adoption of strict measures of containment at the site of production. The necessary expenditure would then have negative economic consequences.

Consequently, a need exists for an industrial strain of *X. campestris* which stably lacks a phytopathogenic nature but which has retained its productivity properties with xanthan gum. In addition, because of regulations and in order to simplify the treatment of the waste derived from separating the xanthan gum from the biomass, it is useful for the strain not to contain a heterologous gene encoding resistance to an antibiotic. Finally, with regard to French and European legislation, it is preferable for the strain obtained to have been constructed by autocloning, which means that it does not contain any DNA elements foreign to its natural genetic

inheritance.

The studies by the inventors have allowed the construction of a strain of *X. campestris* which has the required properties.

Surprisingly, it has been shown, by virtue of the invention, that a bacterium which has become stably nonphytopathogenic, by deletion of a fragment of considerable size which affects several kilobases of genes involved in virulence, is, however, capable of producing xanthan gum.

Even more surprisingly, the modified strain of the invention produces xanthan gum in an amount and a quality in all respects comparable to that produced by the wild-type strain from which the construct was produced.

A subject of the invention is a bacterial strain which has lost the phytopathogenic nature by inactivation of at least one virulence gene, and which has conserved the ability to produce exopolysaccharide.

The bacterial strain according to the invention is advantageously made stably nonphytopathogenic by deletion of at least one gene, advantageously at least two genes, preferably at least three genes, of the *hrp* or *hrc* gene group, and preferably 5 to 9 genes of the *hrp* or *hrc* gene group.

The expression "stably lacks a

phytopathogenic nature" is intended to mean that this character is conserved after a number of cell cycles of at least 20 generations, advantageously of at least 30 generations, preferably of at least 40 generations.

Among the bacteria which have lost their phytopathogenic nature and which can advantageously be used for industrial production of exopolysaccharide, mention may be made in particular of the following genera: *Erwinia, Pseudomonas, Ralstonia* and *Xanthomonas*.

A subject of the invention is in particular a *Xanthomonas* strain which essentially stably lacks a phytopathogenic nature and which has substantially conserved the ability to produce exopolysaccharide.

The expression "essentially nonphytopathogenic" is intended to mean the absence of spreading lesions and/or withering on leaves of host crucifer plants, in particular cabbage (*Brassica oleracera*), after at least 15 days following inoculation of the leaf by injuring the midrib.

Advantageously, the *Xanthomonas* strain is of the species *campestris*, in particular *pv. campestris*.

The inactivation of said gene(s) is preferably obtained by deletion of at least 1 kb, preferably at least 3 kb, advantageously at least 5 kb, in the *hrp* or *hrc* gene group, preferably 9 kb and

possibly ranging up to 40 kb in the *hrp* or *hrc* gene group.

In a preferred embodiment, the essentially nonphytopathogenic strain of *Xanthomonas*, in particular *campestris*, according to the invention is obtained by deletion of the *hrpA1* to *hrpC2* genes of a phytopathogenic wild-type strain of *Xanthomonas campestris* pv *campestris*.

The xanthan gum produced by the strains of *Xanthomonas* of the invention is a xanthan gum substantially identical to that produced by the wild-type species, namely it has substantially the same molecular weight distribution, and also the same degree of modifications, in particular degrees of acetylation and of pyruvylation.

A subject of the invention is also a method for preparing a strain as defined above, characterized in that it is obtained by homologous recombination with a plasmid comprising a deletion of all or part of the *hrp* or *hrc* genes.

A subject of the invention is also a method for preparing bacterial exopolysaccharide, in particular xanthan gum, characterized in that a bacterial strain, where appropriate of the *Xanthomonas* genus, preferably of the species *Xanthomonas campestris*, as defined above is cultured under

conditions which allow the production of exopolysaccharide in the fermentation medium.

The following examples illustrate the construction of strains of *Xanthomonas campestris* which correspond to the characteristics of the invention.

In these examples, the construction was carried out using a strain of *Xanthomonas campestris pv campestris* obtained by screening xanthan gum.

It goes without saying that other strains of *Xanthomonas* and also of exopolysaccharide-producing bacteria which belong to a different genus and which are accessible to those skilled in the art, may be used as starting raw material for producing nonphytopathogenic strains, in accordance with the general knowledge of the technical field in question and with the indications given hereinafter, in particular with reference to the portions of sequences reported when the strain belongs to the species *Xanthomonas campestris*.

In order to understand the examples, reference will be made to the attached figures in which:

- figure 1 diagrammatically represents the strategy for construction of derivatives of the strain of *X. campestris* RPA-BIOCAT826, which carry an *hrp* gene deletion.

The organization of the *hrp* genes in *X. campestris pv vesicatoria* is described by Fenselau and Bonas (1995, Mol. Plant Microbe Interact. 8 (6), 845-854) and by Fenselau *et al.*, (1992, Mol. Plant Microbe Interact. 5, 390-396) and is partly available in Genebank under the accession number U 33548. The homologous regions cloned from the RPA-BIOCAT826 strain are represented, as is the name of the plasmids in which they were cloned. The restriction map of the *hrp* region of *X. campestris* pv *campestris* is published by Arlat *et al.*, 1991, Mol. Plant Microbe Interact 4: 593-601, and is completed by the results given in examples 1 to 4. The *ΔhrpA1-C2* deletion carried by the pRPA-BCAT140 plasmid described in the examples was introduced into the genome by double homologous recombination;

- figure 2 represents the hybridization signals obtained by Southern Blot with the HRPB5 probe described below and the genomic DNAs of the RPA-BIOCAT826 strain and of two derivatives of this strain which have integrated the *ΔhrpA1-C2* deletion. The position of the size marker bands was reported by comparison with the distance of migration on the gel stained with ethidium bromide before transfer. These sizes are expressed in kilobases.

- figure 3 represents the hybridization

signals obtained by Southern Blot with the HRPC2 probe described below and the genomic DNAs of the RPA-BIOCAT826 strain and of 5 derivatives of this strain which have integrated the *ΔhrpA1-C2* deletion. The position of the size marker bands was reported by comparison with the distance of migration on the gel stained with ethidium bromide before transfer. These sizes are expressed in kilobases.

Materials and methods

Unless otherwise specified, the techniques used are conventional molecular biology and microbiology techniques known to those skilled in the art, as described, for example, by Ausubel *et al.*, 1987 (Current Protocols in Molecular Biology, John Wiley and Sons, New York; Maniatis *et al.*, 1982, Molecular Cloning: a laboratory manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) and Coligan *et al.*, 1997 (Current Protocols in Protein Science, John Wiley & Sons, Inc.).

1. Starting strain

The RPA-BIOCAT826 strain is derived from the collection of Rhodia Chimie (Melle factory, RTAM) and was selected for its white morphological appearance instead of the usual yellow appearance. The RPA-BIOCAT1016, 1017, 1019 and 1021 strains were deposited at the CBS under the respective numbers CBS 101940,

CBS 101941, CBS 101942, CBS 101943 and CBS 101944.

2. MSX culture medium

The MSX medium used for culturing *Xanthomonas* contains: 0.2 g/l of yeast extract; 1.2 g/l of NH_4NO_3; 7.3 g/l of K_2HPO_4; 0.25 g/l of $MgSO_4.7H_2O$; 1 g/l of glucose and 15 g/l of Bacto-Agar for the agar medium; 10 g/l of glucose for the liquid medium. The magnesium sulfate and the glucose are sterilized separately and added extemporaneously. The pH of the medium is equilibrated at pH 7.2, before sterilization, with sulfuric acid diluted to 10%.

The genomic DNA preparations were produced from young liquid cultures in MSX (OD660 less than 0.4). After centrifugation of 40 ml of culture, the cell pellet is taken up in 11.9 ml of TE buffer (Current Protocols in Molecular Biology, John Wiley and Sons, New York) and 630 μl of 10% SDS (sodium dodecyl sulfate), and then 63 μl of proteinase K at 20 mg/ml are added. After incubation for 1 h at 37°C, 2.1 ml of 5M NaCl are added, followed by 1.7 ml of 10% CTAB in a 0.7M NaCl solution, and the entire mixture is incubated for 10 minutes at 65°C. After a first extraction with an equivalent volume of a chloroform/isoamyl alcohol (24:1) mixture followed by a second extraction with an equivalent volume of a phenol/chloroform/isoamyl alcohol (25:24:1) mixture, the supernatant is added to

0.6 volume of isopropanol. After centrifugation (5 min at 10 000 rpm), the pellet obtained is washed in 70% ethanol and then dried before being taken up in at least 2 ml of TE, to which 25 μl of a 5 mg/ml RNAse solution are added. After incubation for 1 h at 37°C, an extraction with phenol/chloroform/isoamyl alcohol is carried out and the DNA from the supernatant is precipitated by adding 0.1 volume of 3M sodium acetate and 2.5 volumes of ethanol. The pellet obtained after centrifugation for 5 minutes at 14 000 rpm is washed with 70% ethanol, dried and then resuspended in at least 0.5 ml of TE.

EXAMPLE 1:

Cloning of the hrpC2 region of RPA-BIOCAT826

The region targeted was amplified by PCR starting with the genomic DNA of the RPA-BIOCAT826 strain using the primers XcC2.3 (SEQ ID No. 1) and XcC2.4 (SEQ ID No. 2). The genomic DNA of the RPA-BIOCAT826 strain was extracted and used in a PCR reaction containing 100 ng of genomic DNA, 40 pmol of each primer, 0.2 mM dNTP and 1.25 U of Pwo polymerase (Boehringer Mannheim) in a final volume of 50 μl of the buffer for this enzyme. After incubation for 5 min at 95°C, the mixture first underwent 30 cycles comprising incubation for 1 min at 94°C, then 1 min at a temperature ranging from 63°C to 48°C (in steps of

0.5°C per cycle) and 1 min at 72°C, then 15 cycles comprising incubation for 1 min at 94°C, followed by 1 min at 48°C, and one minute at 72°C and, finally, 10 min at 72°C. The amplification product, which was close to 1.2 kb in size, was purified by migration on agarose gel and then using the Qiaex kit (Quiagen). It was then cloned into the pZERO-1 vector (Invitrogen BV) opened with EcoRV. After transformation of the *E. coli* strain JM110, a clone harboring a plasmid which had integrated the 1.2 kb fragment was selected. This plasmid was named pRPA-BCAT91 and the insert which it contained was sequenced (Genome Express, Grenoble, France). The sequence obtained (SEQ ID No. 3) was aligned with the sequence of the hrpC2 gene of *X.* campestris pv vesicatoria (Fenselau et al., 1992, Molecular Plant Microbe Interactions, 5: 390-396). 87% identity was found over the 1188 bp representing 61% of the *hrpC2* gene. The amino acid sequence deduced from the nucleotide sequence shows a percentage identity of 92% compared to the equivalent portion of the sequence of the *HrpC2* protein of *X. campestris pv vesicatoria*.

EXAMPLE 2:

Cloning of the HrpA region of RPA-BIOCAT826

This region was cloned by screening a partial genomic library of the RPA-BIOCAT826 strain using a nucleotide probe corresponding to the equivalent region

of the *X. campestris pv vesicatoria* strain. This region is available in a plasmid named pL3o, which contains a 6.6 kb EcoRV insert encompassing the *hrpB8* and *hrpA1* genes of *X. campestris pv vesicatoria* (Fenselau *et al.*, 1992, Molecular Plant-Microbe Interactions, 5: 390-396).

The HRPA1 probe was prepared by PCR using the primers XcvA15 (SEQ ID No. 4) and XcvA18 (SEQ ID No. 5), each at 40 pmol, the pL3o plasmid matrix (40 ng), 0.2 mM dNTP and 1.25 U of Pwo polymerase (Boehringer Mannheim) in a final volume of 50 μl of the buffer for this enzyme. After incubation for 5 min at 95°C, the mixture underwent 30 cycles comprising a sequence of 30 seconds at 94°C, 1 min at 55°C and 1.5 min at 72°C. After a final incubation of 10 min at 72°C, the 664 bp amplification product was purified on agarose gel and then with the Quiaex kit (Quiagen).

Approximately 10 μg of genomic DNA of the RPA-BIOCAT826 strain were digested with 100 units of EcoRI for 16 h at 37°C. The conventional Southern Blot technique was then used in order to determine the size of the EcoRI fragment which hybridized with the HRPA1 probe described above. After migration on agarose gel of the EcoRI digestion above, transfer onto a Hybond N+ membrane (Amersham) using hybridization at 55°C for 19h in an aqueous hybridization solution (0.5% SDS; 6% SSC;

0.25% of powdered skimmed milk) with the HRPA1 probe labeled with phosphorus 32 using the Ready-To-Go kit (Pharmacia Biotech) according to the manufacturer's indications, and washing at 55°C with a solution of 0.2 SSC and 0.1% SDS, the membrane was autoradiographed for 19 h at -80°C. Development of the film revealed a hybridization signal close to 7.3 kb in size.

A partial genomic library of the RPA-BIOCAT826 strain was therefore produced by digesting 100 μg of genomic DNA of this strain with 1 000 units of the EcoRI enzyme for 20 h at 37°C. After migration on agarose gel, the region corresponding to the fragments between 7 and 8 kb in size was cut out and the DNA extracted from the gel by electroelution in a dialysis bag (Spectra/Por membranes from Spectrum Medical Industries, Inc.). After precipitation with ethanol, the DNA was ligated in a final volume of 10 μl to the pBlueScript II SK vector (Stratagene), opened beforehand with the EcoRI enzyme and then dephosphorylated with shrimp alkaline phosphatase (United States Biochemicals). After incubating the ligation mixture for 14 h at 16°C, a tenth of the mixture was used to transform *E. coli* DH5alpha cells by electroporation. Approximately 3 000 transformants were analyzed by hybridization of colonies transferred onto nylon membrane, using the HRPA1 probe. Twelve colonies

giving a positive hybridization signal were purified on LB agar medium containing 100 μg/ml of ampicillin. The plasmids of twelve purified colonies were extracted and EcoRI digestions of these plasmids were analyzed by Southern blot with the HRPA1 probe in order to confirm the presence of an approximately 7.3 kb fragment which hybridized with this probe. After restriction analysis with various enzymes, a 2.7 kb SacII fragment and a 1.6kb SacII fragment were subcloned into the pBlueScript II SK vector opened with SacII, to give the pRPA-BCAT135 and pRPA-BCAT134 vectors, respectively. These two vectors were partially sequenced (Genome Express, Grenoble) and this revealed the presence of a 1818 bp open reading frame (SEQ ID No. 6), the deduced peptide sequence of which exhibits 85% identity with the HrpA1 protein of *X. campestris pv vesicatoria* (Fenselau *et al.*, 1992, Molecular Plant-Microbe Interactions, 5: 390-396).

EXAMPLE 3:

Construction of strains derived from RPA-BIOCAT826, containing a *ΔhrpA1-C2* deletion

The *ΔhrpA1-C2* deletion was constructed *in vitro* by cloning, into the pJQ200SK plasmid (Quandt and Hynes, 1993, Gene 127: 15-21), a fragment of pRPA-BCAT134 and a fragment of pRPA-BCAT91 (cf. figure 1). The pRPA-BCAT91 plasmid was opened with NcoI and then

treated with polymerase I (Klenow fragment) for 15 min at 30°C in the presence of 25 μM of dNTP. After extraction with phenol/chloroform/isoamyl alcohol and then precipitation with ethanol, the sample was taken up in 40 μl of water in order to be treated with 20 units of XbaI at 37°C followed by 20 units of ApoI at 50°C. The approximately 1.2 kb fragment was then separated by gel and recovered with the Quiex II kit (Quiagen). The approximately 1.3 kb RsaI-SacII fragment of pBCAT134 was purified in an identical way. These two fragments were ligated to the pBlueScript II SK vector opened with the SacII and XbaI enzymes, to give the pRPA-BCAT139 plasmid. An approximately 2.5 kb SacI-XbaI fragment carrying the *ΔhrpA1-C2* deletion could then be extracted from this plasmid so as to be cloned into the pJQ200KS plasmid opened with the SacI and XbaI enzymes. The resulting plasmid was named pRPA-BCAT140. It is a plasmid which is nonreplicative in *X. campestris*, which carries the gentamycin resistance marker for selecting the clones of *X. campestris* which have integrated the plasmid by homologous recombination, and which carries the positive selection marker sacB for selecting the clones which have eliminated the gentamycin resistance marker following a second homologous recombination event.

The pRPA-BCAT140 plasmid was introduced into

the RPA-BIOCAT826 strain by conjugation. To do this, 40 μl of a culture in the exponential phase of the DH5alpha strain harboring pRPA-BCAT140, 40 μl of a culture in the exponential phase of the HB101 strain harboring the pRK2013 plasmid (Ditta *et al.*, 1980, Proc. Natl. Acad. Sci. USA 77: 7347-7351) and 40 μl of a culture of the RPA-BIOCAT826 strain in the exponential phase, in an MSX medium, were mixed on MSX agar medium. After incubation for 24 h at 30°C, the clones of *X. campestris* which had integrated the pRPA-BCAT140 plasmid were purified twice consecutively on an MSX agar medium containing 15 μg/ml of gentamycin. Eight clones were then plated out over a surface of approximately 1 cm^2 on an MSX agar medium containing 5% sucrose. After incubation for 72 h at 30°C, colonies were isolated by two successive purifications on MSX agar medium. Approximately 300 colonies were then subcultured on MSX agar medium containing 15 μg/ml of gentamycin in order to identify the gentamycin-sensitive clones (from 90 to 100% of the clones depending on the assays). About forty of these clones were then analyzed by Southern Blot using EcoRI-BamHI digestion of their genomic DNA and the HRPA1 probe. Approximately 25% of the clones exhibited a signal which was different from that of the wild-type RPA-BIOCAT826 strain and coherent with integration of the

ΔhrpA1-C2 deletion. Five clones were selected for the remainder of the experiments: RPA-BIOCAT strains 1016, 1017, 1019, 1021 and 1022.

EXAMPLE 4:

Characterization by Southern Blot of the strains derived from RPA-BIOCAT826, containing a *ΔhrpA1-C2* deletion

The RPA-BIOCAT strains 1016, 1017, 1019, 1021 and 1022 were characterized by analyzing the hybridization profiles of EcoRI, BamHI and EcoRI-BamHI digestions of genomic DNA, with the HRP3'A1, HRPB5 and HRPC2 probes.

The HRP3'A1 probe was obtained by purifying the 1.6 kb SacII fragment of the pRPA-BCAT134 plasmid by migration on gel and using the Quiaex kit.

The HRPC2 probe was obtained by purifying the 1.2 kb EcoRI-XbaI fragment of the pRPA-BCAT91 plasmid by migration on gel and using the Quiaex kit.

The HRPB5 probe was obtained by purifying the 1.5 kb BamHI fragment of the pRPA-BCAT129 plasmid by migration on gel and using the Quiaex kit. Sequencing of this insert revealed, in particular, an open reading frame (SEQ ID No. 7), the deduced peptide sequence of which exhibits 77% identity with the HrpB5 protein of *X. campestris pv vesicatoria* (Fenselau *et al.*, 1995, Mol. Plant-Microbe Interactions, 8: 845-854). The pRPA-

BCAT129 plasmid was obtained by cloning the BamHI genomic DNA fragments of the RPA-BIOCAT826 strain, which are between 1.3 and 1.9 kb in size, into the pBlueScriptIISK vector and screening the colonies with an HRPB probe in a manner similar to that described in example 2. The HRPB probe was obtained by PCR using the primers RST2 and RST3 (Leite *et al.*, 1994, Appl. Environ. Microbiol. 60: 1068-1077) and the pB10g plasmid matrix (U. Bonas, personal communication). The pB10g plasmid corresponds to the pBluescriptKS plasmid into which the 7.3 kb BamHI fragment containing the *hrpB* region and the *hrpA1* gene of *Xanthomonas campestris pv vesicatoria* (Fenselau et al., 1995, Mol. Plant-Microbe Interactions, 8: 845-854) is cloned. The PCR reaction was carried out with 40 pmol of each primer, 50 ng of pB10g, 0.2 mM dNTP and 1.25 U of Pwo polymerase (Boehringer Mannheim) in a final volume of 50 μl of the buffer for this enzyme. After incubation for 5 min at 95°C, the mixture first underwent 24 cycles comprising incubation for 30 seconds at 95°C, then 40 seconds at a temperature ranging from 70°C to 63°C (by steps of 0.3°C per cycle) and 1 min at 72°C, and then 6 cycles comprising incubation for 30 seconds at 95°C, followed by 40 seconds at 63°C and one minute at 72°C and, finally, 5 min at 72°C. The approximately 840 bp fragment was then purified on agarose gel and

using the Quiaex kit (Quiagen).

The Southern Blot analysis was carried out by labeling the probes using the "Megaprime DNA labelling system" kit (Amersham) according to the instructions provided. After migration on agarose gel, the genomic DNA digestions were transferred onto Hybond N+ membranes (Amersham) according to the indications provided, and then incubated in the hybridization solution composed of a 0.5M phosphate buffer and of 7% SDS (115 ml of 1M Na_2HPO_4, 84.6 ml of [lacuna] M NaH_2PO_4, 200 ml of H_2O and 28 g of SDS). The labeled probes are incubated for 5 min at 100°C and then for 5 min at room temperature, before being diluted in 12 ml of hybridization solution and incubated for 5 min at 100°C. This mixture is then brought into contact with the membranes for 6 to 20 h at 65°C. The latter are then washed for 10 to 15 minutes in a 0.1 M phosphate buffer containing 1% of SDS (42.3 ml of 1M Na_2HPO_4, 57.7 ml of 1M NaH_2PO_4, 900 ml of H_2O and 10g of SDS) and then exposed.

The results obtained with the HRPB5 probe (figure 2) show, for the RPA-BIOCAT826 strain, a hybridization signal at approximately 4.8 kb with the EcoRI digestion and a signal at 1.6 kb with the BamHI digestion and the EcoRI-BamHI digestion. These results are in agreement with the mapping by Arlat *et al.*

(Molecular Plant-Microbe Interactions, 1991, 4: 593-601) and the location of the *hrpB5* gene described above. None of the RPA-BIOCAT strains studied shows a hybridization signal with HRPB5, which is coherent with integration of the *ΔhrpA1-C2* deletion into the genome of these RPA-BIOCAT strains 1016, 1017, 1019, 1021 and 1022 (figure 2 shows only the hybridization result obtained with the RPA-BIOCAT strains).

The results obtained with the HRPC2 probe (figure 3) show, for the RPA-BIOCAT826 strain, a hybridization signal at approximately 5.5 kb with the EcoRI digestions and a signal at approximately 2.6 kb with the EcoRI-BamHI digestion. These results are in agreement with the mapping by Arlat *et al.* (Molecular Plant-Microbe Interactions, 1991, 4: 593-601), the organization of the *hrp* genes in *X. campestris pv vesicatoria* (Fenselau *et al.*, 1992, Molecular Plant-Microbe Interactions, 5: 390-396) and the location of the *hrpB5* gene described above. The results obtained with the RPA-BIOCAT strains 1016, 1017, 1019 and 1021 show a signal between 7 and 8 kb with the BamHI digestions and a signal at 4.4 kb with the EcoRI-BamHI digestions. Given the mapping shown in figure 1, these results are coherent with integration of the *ΔhrpA1-C2* deletion into the genome of the RPA-BIOCAT strains 1016, 1017, 1019, 1021 and 1022.

Finally, the results obtained with the HRP3'A1 probe show, for the RPA-BIOCAT826 strain, a hybridization signal at approximately 7.3 kb for the EcoRI-BamHI digestion. With the RPA-BIOCAT strains 1016, 1017, 1019 and 1021, this hybridization signal is at 4.4 kb, which is coherent with integration of the *ΔhrpA1-C2* deletion into the genome of these strains.

EXAMPLE 5:

Virulence of the strains derived from RPA-BIOCAT826, containing an HrpA1-C2 deletion

The virulence tests were carried out on cabbage plants (*Brassica oleracera* var. *captiva* cultivar *Siria*), the seeds of which were obtained from Clause Semences (av. Lucien Clause, 91221 Brétigny-sur-Orge, France). The plants were cultivated in a climatic cell according to the following parameters: 14 hours at 25°C, 55% humidity, saturating light intensity (4 000 W/m); 10 hours at 25°C, 60% humidity. They were infected at the 2-leaf stage, i.e. approximately 13 days after sowing. For each strain tested, 8 plants were used, piercing the first leaf in the midrib of the terminal portion using an infected toothpick. The toothpick was contaminated by immersing its tip in a 2-day culture of the strain studied in MSX medium (approximately 10^8 bacteria/ml). The negative controls consisted of a mixture of reference strains of

X. campestris pv vesicatoria (B229RI strain = RPA-BIOCAT381 and B230RII strain = RPA-BIOCAT382), phytopathogenic on peppers, isolated at Clause Semences. The positive controls consisted of a mixture of reference strains of *X. campestris pv campestris* (2963 strain = RPA-BIOCAT379 and 63C2AM strain = RPA-BIOCAT380), phytopathogenic on cabbages, isolated at Clause Semences. The symptoms (V-shaped yellow lesions) were read and measured 12 and 14 days after infection. For each plant, a score was given corresponding to the following: 0, no symptoms, 1, depigmentation located close to the point of infection; 2, necrosis less than 0.5cm^2; 3, necrosis of 0.5 to 1.5 cm^2; 4, necrosis greater than 1.5 cm^2; 5, generalized necrosis of the leaf. The sum of the scores of the 8 plants infected with the same strain is the pathogenicity score for this strain (table 1).

Table 1: Phytopathogenicity of the strains of *Xanthomonas*

STRAINS	D + 12	D + 14
BIOCAT 381/382	0	0
BIOCAT 379/380	32	39
BIOCAT826	28	34
BIOCAT 1016	4	4
BIOCAT 1017	5	5
BIOCAT 1019	2	3
BIOCAT 1021	1	1
BIOCAT 1022	4	5

While the RPA-BIOCAT826 strain causes progressive withering of the leaf, the constructed

strains, caused, at most, localized necrotic withering, which reflects a lack of pathogenicity.

EXAMPLE 6:

Production of xanthan by the strains derived from RPA-BIOCAT826, containing an HrpA1-C2 deletion

The xanthan productivity of the strains was assessed by measuring the solids which could be precipitated with isopropanol, contained in 40 ml of culture. After preculturing for 24 hours in MSX, 100 ml of MSX medium in 500 ml erlenmeyer flasks were inoculated with approximately the same number of bacteria (0.4 ml of preculture of OD660 = 0.25). After incubation for 6 days at 30°C with shaking (200 rpm), 40 grams of culture were removed and mixed with 150 ml of isopropanol. After filtration, the fibers recovered were washed twice with 70 ml of isopropanol, before being dried and then weighed as they left the oven. The operation, carried out on three independent cultures of the RPA-BIOCAT826 strain, showed a productivity variability of about 10%. The results obtained with the RPA-BIOCAT826 strain and its *ΔhrpA1-C2* derivatives are given in table 2.

Table 2: Xanthan productivity of RPA-BIOCAT826 and of its *ΔhrpA1-C2* derivatives.

STRAIN	DRY WEIGHT Xt (g)	PRODUCTIVITY (g/g)
BIOCAT826	0.323	8.1×10^{-3}
BIOCAT 1016	0.362	9.0×10^{-3}
BIOCAT 1017	0.366	9.1×10^{-3}
BIOCAT 1019	0.371	9.3×10^{-3}
BIOCAT 1021	0.334	8.4×10^{-3}
BIOCAT 1022	0.329	8.2×10^{-3}

The productivities are expressed in grams of solids extractable with isopropanol per grams of culture.

CLAIMS

1. A bacterial strain which has lost the phytopathogenic nature by inactivation of at least one virulence gene, which has conserved the ability to produce exopolysaccharide and which does not contain DNA foreign to its natural genetic inheritance.

2. The bacterial strain as claimed in claim 1, characterized in that it has been made stably nonphytopathogenic by inactivation of at least one gene, advantageously at least two genes, preferably at least three genes, of the *hrp* or *hrc* gene group.

3. The bacterial strain as claimed in claim 1 or claim 2, characterized in that it has been made stably nonphytopathogenic by inactivation of 5 to 9 genes of the *hrp* or *hrc* gene group.

4. The bacterial strain as claimed in claim 1, characterized in that it is a *Xanthomonas* strain which has lost the phytopathogenic nature by inactivation of at least one virulence gene, and which has conserved the ability to produce exopolysaccharide.

5. The *Xanthomonas* strain as claimed in claim 4, characterized in that it is of the species *Xanthomonas campestris*.

6. The *Xanthomonas* strain as claimed in claim 5, characterized in that it is *Xanthomonas*

campestris pv campestris.

7. The *Xanthomonas* strain as claimed in any one of the preceding claims, characterized in that the inactivation of said gene(s) is obtained by deletion of a region of DNA of at least 1 kb, preferably at least 3 kb, advantageously at least 5 kb, in the *hrp* or *hrc* gene group, and in that it conserves the ability to produce exopolysaccharide.

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- *With the International Search Report.*
- *Before expiry of the period provided for amending the claims, will be republished if such amendments are received.*

For an explanation of the two-letter codes and the other abbreviations, reference is made to the explanations ("Guidance Notes on Codes and Abbreviations") at the beginning of each regular edition of the PCT Gazette.

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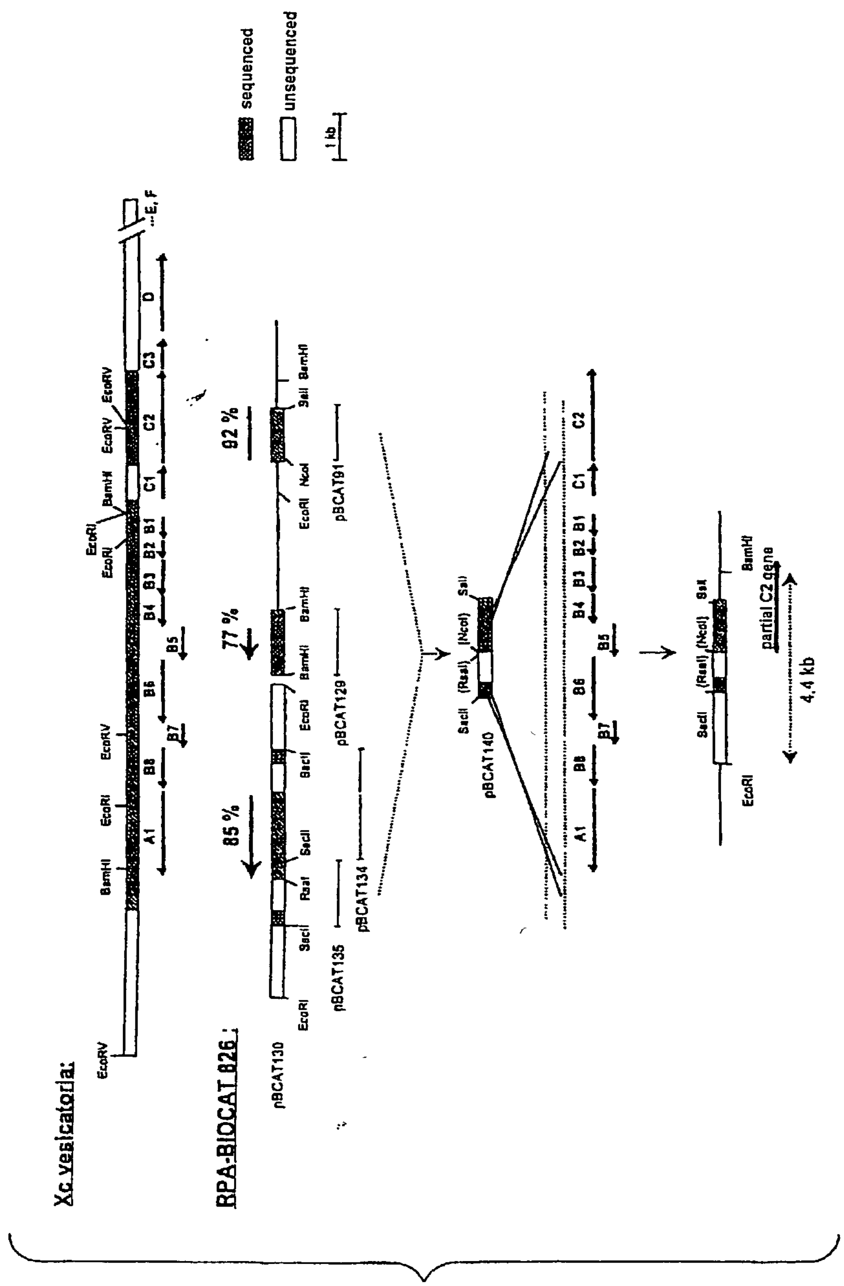
(54) Title: AVIRULENT XANTHOMONAS-CAMPESTRIS STRAINS PRODUCING XANTHAN

(54) Titre: SOUCHES AVIRULENTES DE XANTHOMONAS CAMPESTRIS, PRODUISANT DU XANTHANE

(57) Abstract: The invention concerns a bacterial strain which has lost its phytopathogenic character by inactivation of at least one virulence gene and preserved its capacity for producing exopolysaccharide.

(57) Abrégé: Cette invention concerne une souche bactérienne ayant perdu le caractère phytopathogène par inactivation d'au moins un gène de virulence et ayant conservé la capacité de production d'exopolysaccharide.

1/2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B1
B2
B3
B4
B5
B6
B7
B8
C1
C2
C3
D
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85 %
77 %
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FIG.1

2/2

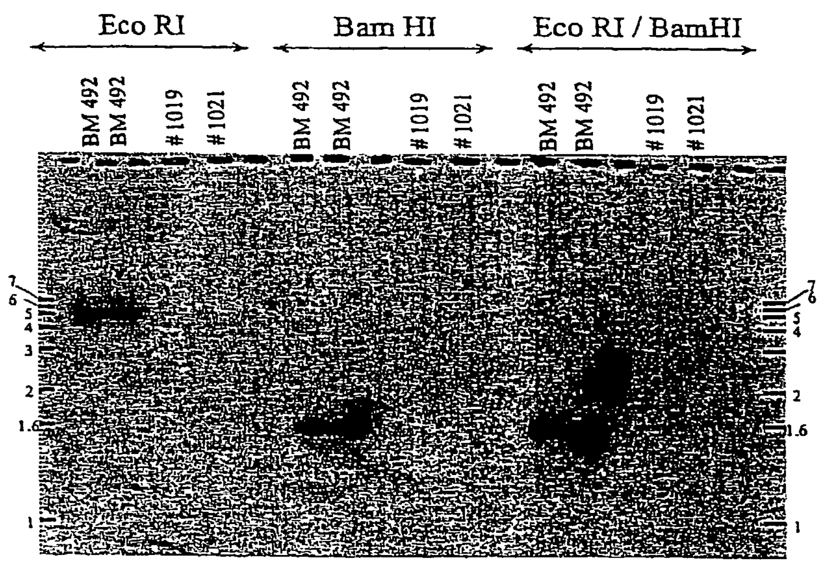
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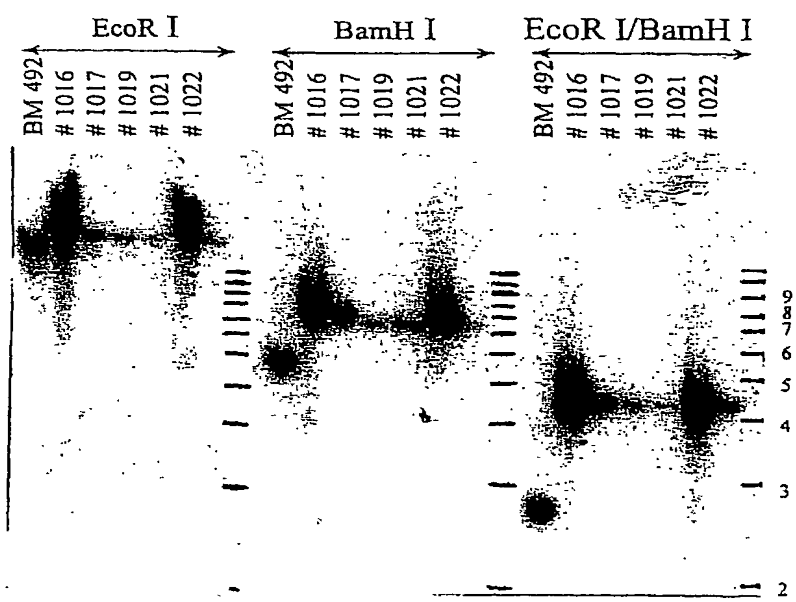
FIG.2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1019
1021
1022
9
8
7
6
5
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3
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FIG.3

R 99073

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Attorney's Docket No.

COMBINED DECLARATION AND POWER OF ATTORNEY FOR UTILITY OR DESIGN PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

AVIRULENT XANTHOMONAS-CAMPESTRIS STRAINS PRODUCING XANTHAN

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application
Number ____________ on ____________
and was amended on ____________ (if applicable).

☒ was filed as PCT international application
Number PCT/FR00/01725 on JUNE 21, 2000
and was amended on DECEMBER 21, 2001 (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §§119 (a)-(d), 172 or 365 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §§119(a)-(d), 172 or 365:				
COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §§119, 172 or 365	
FRANCE	99/07963	22 JUNE 1999	X Yes	No
			Yes	No
			Yes	No
			Yes	No
			Yes	No

10018786 .062602

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

William L. Mathis	17,337	Eric H. Weisblatt	30,505	Bruce T. Wieder	33,815
Robert S. Swecker	19,885	James W. Peterson	26,057	Todd R. Walters	34,040
Platon N. Mandros	22,124	Teresa Stanek Rea	30,427	Ronni S. Jillions	31,979
Benton S. Duffett, Jr.	22,030	Robert E. Krebs	25,885	Harold R Brown III	36,341
Norman H. Stepno	22,716	William C Rowland	30,888	Allen R. Baum	36,086
Ronald L. Grudziecki	24,970	T. Gene Dillahunty	25,423	Brian P. O'Shaughnessy	32,747
Frederick G. Michaud, Jr.	26,003	Patrick C. Keane	32,858	Kenneth B. Leffler	36,075
Alan E. Kopecki	25,813	B. Jefferson Boggs, Jr.	32,344	Fred W. Hathaway	32,236
Regis E. Slutter	26,999	William H. Benz	25,952	Wendi L. Weinstein	34,456
Samuel C. Miller, III	27,360	Peter K. Skiff	31,917	Mary Ann Dillahunty	34,576
Robert G. Mukai	28,531	Richard J. McGrath	29,195	21839	
George A. Hovanec, Jr.	28,223	Matthew L. Schneider	32,814		
James A. LaBarre	28,632	Michael G. Savage	32,596		
E. Joseph Gess	28,510	Gerald F. Swiss	30,113		
R. Danny Huntington	27,903	Charles F. Wieland III	33,096		

and: ______________________________

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Norman H. Stepno, Esquire
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
Alexandria, Virginia 22313-1404

Address all telephone calls to: Teresa Stanek Rea at (703) 838-6638.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	Jérôme PIERRARD
Signature	
Date	March 5, 2002
Residence (City, State, Country)	Saint Didier Au Mont D'Or, FRANCE FRX
Citizenship	FRANCE
Mailing Address	6, chemin des Lavandières
City, State, ZIP, Country	69370 Saint Didier Au Mont D'Or, FRANCE
FULL NAME SECOND INVENTOR, IF ANY	Jean-Luc SIMON
Signature	
Date	
Residence (City, State, Country)	Lille, FRANCE
Citizenship	FRANCE
Mailing Address	266, rue Solférino
City, State, ZIP, Country	59000 Lille, FRANCE

FULL NAME THIRD INVENTOR, IF ANY	Paule CHEVALLEREAU
Signature	
Date	
Residence (City, State, Country)	Melle, FRANCE
Citizenship	FRANCE
Mailing Address	59, rue des Jonchères
City, State, ZIP, Country	79500 Melle, FRANCE
FULL NAME FOURTH INVENTOR, IF ANY	
Signature	
Date	
Residence (City, State, Country)	
Citizenship	
Mailing Address	
City, State, ZIP, Country	
FULL NAME FIFTH INVENTOR, IF ANY	
Signature	
Date	
Residence (City, State, Country)	
Citizenship	
Mailing Address	
City, State, ZIP, Country	
FULL NAME SIXTH INVENTOR, IF ANY	
Signature	
Date	
Residence (City, State, Country)	
Citizenship	
Mailing Address	
City, State, ZIP, Country	
FULL NAME SEVENTH INVENTOR, IF ANY	
Signature	
Date	
Residence (City, State, Country)	
Citizenship	
Mailing Address	
City, State, ZIP, Country	

R 99073

022701-966
Attorney's Docket No.

COMBINED DECLARATION AND POWER OF ATTORNEY FOR UTILITY OR DESIGN PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

AVIRULENT XANTHOMONAS-CAMPESTRIS STRAINS PRODUCING XANTHAN

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application
Number ______________ on ______________
and was amended on ______________ (if applicable).

☒ was filed as PCT international application
Number PCT/FR00/01725 on JUNE 21, 2000
and was amended on DECEMBER 21, 2001 (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

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Signature	
Date	
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Citizenship	FRANCE
Mailing Address	6, chemin des Lavandières
City, State, ZIP, Country	69370 Saint Didier Au Mont D'Or, FRANCE
FULL NAME SECOND INVENTOR, IF ANY	Jean-Luc SIMON
Signature	
Date	6 March 2002
Residence (City, State, Country)	Lille, FRANCE FRX
Citizenship	FRANCE
Mailing Address	266, rue Solférino
City, State, ZIP, Country	59000 Lille, FRANCE

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City, State, ZIP, Country	59000 Lille, FRANCE

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3.00

FULL NAME THIRD INVENTOR, IF ANY	Paule CHEVALLEREAU
Signature	
Date	05/03/2002
Residence (City, State, Country)	Melle, FRANCE FRX
Citizenship	FRANCE
Mailing Address	59, rue des Jonchères
City, State, ZIP, Country	79500 Melle, FRANCE
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